

of OA and IA. Coronal sections were stained with fast green and Safranin O and with specific antibodies for beta-catenin, Smad2/3P, Smad1/5/8P and Runx2. Osteophyte formation and immunohistochemical staining was scored on 11 locations in the joint. In specific experiments, osteophyte formation in IA was compared between wild-type and TNF alpha deficient mice. Since TNF-alpha-induced dickkopf1 has been suggested to be involved in blocking osteophyte formation in arthritis, we have assessed dickkopf1 expression arthritis in wild type and TNF alpha knock out mice by RT-QPCR.

Results: In all OA and IA models, osteophytes that form through endochondral bone formation were detected. Strikingly, intramembranous bone apposition, localized on the tibial and femoral bone collar, was observed in the IA but not in the OA models. Immunohistochemistry of factors reported to be important in cartilage and bone formation showed that expression of beta-catenin was found in hypertrophic chondrocytes in osteophytes undergoing terminal differentiation but not at sites that showed early osteophyte formation. Expression of Runx2 was detected in more than 50% of the cells at sites that showed early osteophyte formation in both IA and OA models. Interestingly, at the same sites, IA and OA models presented high Smad1/5/8P expression but only the OA models showed also high Smad2/3P expression. No clear Smad2/3P expression was found in the IA models.

In addition, TNF-alpha deficiency did not result in increased osteophyte formation. Moreover, severe arthritis in IA models was associated with increased osteophyte formation but not with concomitantly decreased dickkopf-1 expression.

Conclusions: Intramembranous bone apposition was only observed in IA models, indicating that bone formation in IA and OA models is dissimilar. Absence of TNF-alpha did not result in increased osteophyte formation, showing that neither TNF-alpha itself nor TNF-alpha-induced dickkopf1 blocked osteophyte formation in the models studied. Runx2 and TGF-beta superfamily signaling was observed at site of early osteophyte formation. In IA, Smad1/5/8P was expressed but Smad2/3P was absent. In OA both Smad pathways were clearly activated. This suggest that expression of Smad1/5/8P is related to osteophyte formation by the endochondral route while activation of Smad2/3P blocks intramembranous bone apposition. The balance of activated Smad2/3 and Smad1/5/8 pathways appears to be involved in regulation of osteophyte formation in IA and OA.

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ATTENUATION OF OSTEOARTHRITIS PROGRESSION BY REDUCTION OF EXPRESSION OF DISCOIDIN DOMAIN RECEPTOR 2

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Purpose: The objective of this study is to investigate if reduction of DDR2 expression attenuates OA progression induced either by a genetic mutation or by a non-genetic factor.

Methods: We generated double-heterozygous mutant mice, type XI collagen- and Ddr2-haploinsufficient mice in this study. We also performed microsurgery on knee joints of Ddr2-deficient mice. We anticipate that the reduction of the Ddr2 expression will attenuate the OA progression in mouse knee joints induced either by deficiency of type XI collagen or by the microsurgery. A serial paraffin sections were prepared from the knee joints of the mice at different stages of the disease. The articular cartilages of the mouse knee joints were examined by Safranin O/Fast green staining. Morphological conditions of the articular cartilages of the mouse knee joints were evaluated by a modified Mankin score system.

The Ddr2 and Mmp13 protein expression profiles were examined by immunohistostaining in the mouse knee joints.

Results: Results from histology showed that the OA pathological progression was delayed in knee joints of double-heterozygous mutant mice and the OA pathological progression induced surgically in knee joints of Ddr2-deficient mice was also delayed. The expression of Ddr2 and Mmp-13 was reduced in the knee joints of the double-heterozygous mutant mice, compared with that in the wild-type littermates at each time point when we examined.

Conclusions: Taken all above-mentioned results, it suggests that the reduction of Ddr2 expression attenuates the OA progression in mouse OA knee joints.

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A POSSIBLE ROLE OF HTRA1, A SERINE PROTEASE, IN PATHOGENESIS OF OSTEOARTHRITIS

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Purpose: The objective of this study is to investigate a possible role of HTRA1, a serine protease, in pathogenesis of OA by use of four different mouse OA models.

Methods: In this study we examined the protein expression profile of Htra1 in the knee and temporomandibular (TM) joints of four mouse OA models; two of the models had genetic forms of OA and two had non-genetic forms of OA. The level of Htra1 mRNA in the articular cartilage of knee joints, taken from one of the genetically mutated models, was also examined. This was done with the purpose of determining whether the increased expression of Htra1 protein was paralleled by an up-regulated expression of Htra1. Finally, we examined whether the increased expression of Htra1 resulted in the disappearance of type VI collagen, which is a good indicator of the integrity of the pericellular matrix of chondrocytes, and thus led to the elevated expression of Ddr2 in the knee joints of the mutant mice. Paraffin sections were prepared from the knee and temporomandibular (TM) joints of four mouse OA models; two of the models had a genetic mutation (type IX collagen-deficient and type XI collagen-haploinsufficient) and two were surgically induced (destabilization of the medial meniscus of knee joint and discectomy). The Htra1 protein expression profiles of the prepared sections were examined by immunohistostaining. The level of Htra1 mRNA in the articular cartilage taken from the knee joints of one of the genetically mutated OA models was determined by real-time PCR. Double immunohistostaining was used to examine the expression of co-localization of Htra1 with type VI collagen and Htra1 with discoidin domain receptor 2 in the articular cartilage of knee joints from the genetically mutated OA model.

Results: The expression of Htra1 was found to be increased in the knee and TM joints of the four mouse OA models at early stages of the disease. An examination of the knee joint of a mutant mouse indicated an 8-fold increase in the level of Htra1 mRNA, when compared to the levels observed in the knee joints of its wild-type littermates. Pericellular type VI collagen was not present in chondrocytes expressing Htra1. Meanwhile, expression of Htra1 was associated with the expression of Ddr2 in the chondrocytes.

Conclusions: Results indicate that Htra1 may disrupt the pericellular matrix network, resulting in the alteration of chondrocyte metabolisms. This eventually leads to OA.